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Formulation and Evaluation of *Tamarindus indica* Extract Loaded Transfersomal Gel for Anti-inflammatory Effect

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ABSTRACT

This study aimed to develop a novel transferosomal gel formulation loaded with *Tamarindus indica* extract for its anti-inflammatory potential. Transfersomes, being ultra-flexible vesicles, were chosen to encapsulate the extract due to their ability to penetrate deep into the skin layers, enhancing drug delivery. The formulation was optimized using a varying phospholipid concentration, ethanol content, and sonication time as independent variables. The optimized transferosomal gel exhibited desirable physicochemical properties such controlled drug release. The transferosomal gel was evaluated for its anti-inflammatory effect using carrageenan-induced paw edema model in rats. The results demonstrated a significant reduction in paw volume and inflammation in rats treated with the *Tamarindus indica* extract loaded transferosomal gel compared to control groups. Additionally, the transferosomal gel exhibited good skin compatibility and stability upon storage. Thus, the developed *Tamarindus indica* extract loaded transferosomal gel holds promise as a potent anti-inflammatory formulation for topical application, offering a potential alternative to conventional anti-inflammatory therapies.

Keywords: Transdermal; Extended; Bioactive; Transfersomes; Hydrophilic; Anti-inflammatory; Hydrophobic; Ultra-flexible vesicles.

1. Introduction

1.1. Transfersomes

The goal of research has switched from traditional to innovative drug delivery systems (NDDS) in an effort to improve patient compliance and therapeutic efficacy in the modern context (Figure 1 Transfersomes). Transdermal patches, microsomes, and other drug delivery methods with higher therapeutic efficacy have all been developed thus far. The transdermal route is regarded as the safest and most effective in state-of-the-art drug delivery systems since it offers several benefits such a consistent and prolonged duration of action, avoidance of first pass metabolism, fewer adverse effects, etc. [1],[2].

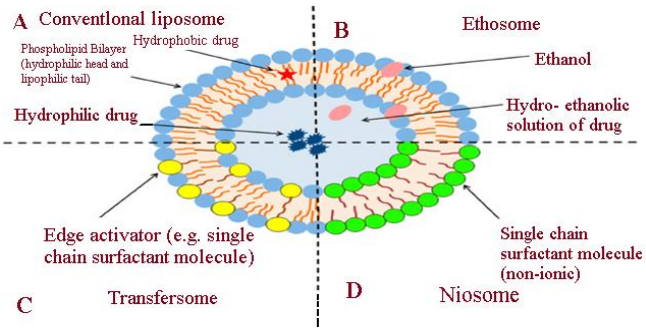

Figure 1. Transfersomes [1]

2. Material and Methods

2.1. Processing of the Plant Material

The plant was taken from the Gwalior medicinal garden at ITM University, dried, and then powered. The extract was then obtained by using this powder further.

2.2. Extraction of plant material

Tamarindus indica leaves were gathered in the Gwalior area (Madhya Pradesh). After being dried in the shade, leaves were ground into a coarse powder using a machine grinder. The powder was extracted using ethanol as the solvent in a Soxhlet extractor after being run through screen No. 40. After that, the extracts were chilled.

2.2.1. Phytochemical study of *Tamarindus indica* extract

Tests for alkaloids: Dragendorff's test is one of the alkaloids tests. An orange-red precipitate was generated when 1 milliliter of Dragendorff's reagent was added to 2 milliliters of extract, signifying the presence of alkaloids.

Tests for flavonoids: The Alkaline Reagent Flavonoid Test. To two milliliters of extract, two to three drops of sodium hydroxide were added. When a few drops of diluted HCl were added, the initially bright yellow color eventually became colorless, suggesting the presence of flavonoids.

Tests for proteins: The Biuret test is a protein test. One milliliter of extract was mixed with two drops of 3% copper sulphate and a few drops of 10% sodium hydroxide; the production of a violet or red color indicates the presence of proteins.

Test for carbohydrates: The Molish test is a test for carbohydrates. To two milliliters of extract, a few drops of an alcoholic a-naphthol solution were added. Afterwards, a few concentrated H_2SO_4 droplets were applied to the test tube walls. Carbohydrates were present because a violet colored ring formed at the intersection of two liquids.

Tests for glycosides: The Keller Killiani test is a glycoside test. Two milliliters of the extract were combined with a 0.5 milliliter solution of glacial acetic acid and two to three drops of ferric chloride. Afterwards, 1 mL of concentrated H_2SO_4 was added to the test tube's walls. The presence of cardiac glycosides was shown by the intense blue color that appeared at the interface of two liquids.

Tests for saponins: Five milliliters of extract were placed in a test tube together with a drop of Na_2CO_3 solution. It was given a good shake and then allowed to rest for five minutes. The production of foam suggested the presence of saponins.

Test for terpenoids: Horizon test - One milliliter of extract was mixed with two milliliters of trichloroacetic acid. The production of a crimson precipitate indicated the presence of terpenoids.

Test for steroids: Salkowski test: a measure for steroids. Concentrated H_2SO_4 was applied along the test tube walls after the test extract was agitated with chloroform; this caused a red color to develop, signifying the presence of steroids.

2.3. Study of drug-excipient interactions

A Fourier Transform Infrared Spectroscopy (FT-IR) spectrophotometer was used to analyze the medication and its excipients. The drug's interaction with the excipients was identified by the interpretation of I.R. Spectrums.

2.4. Solubility Studies

Solubility is the term used to describe the spontaneous interaction of two or more substances to generate a homogenous molecular dispersion.

2.5. Preparation of phosphate buffer pH 6.4

The phosphate buffer, pH 6.4, was created by dissolving 2.5 grams of disodium hydrogen phosphate, 1.36 grams of potassium dihydrogen phosphate, and 7.02 grams of sodium chloride in 1000 milliliters of distilled water. Adjust the pH as necessary.

2.6. Formulation of Transfersomes Gel

2.6.1. Formulation of Transfersomes

Every transfersome preparation process consists of two phases. Sonicated vesicles are first homogenized by extrusion over a membrane filter, resulting in the preparation of a thin film. Using a rotary evaporator, the combination of phospholipids and surfactant—the materials that form vesicles—were mixed in a volatile organic solvent. The organic solvent then evaporated above the lipid transition temperature. Under a vacuum, the last remnants of the solvent were eliminated overnight. The lipid films that had been deposited were then hydrated using buffer (6.4) by rotating at 60 rpm/min for one hour at the appropriate temperature. At room temperature, the resultant vesicles swelled for two hours. Then probe sonicated for 30 minutes at room temperature in order to create the tiny vesicles. By manually extruding the sonicated vesicles through a membrane filter, they were homogenized.

Table 1. Formulation of Transfersomes

S. No.	Chemical Name	Use (w/w)
01	Ether	10 ml
02	Soya Lecithin	500 mg
03	Cholesterol	200 mg
04	Phosphate Buffer 6.4 pH	10 ml
05	Extract of *Tamarindus indica*	500 mg

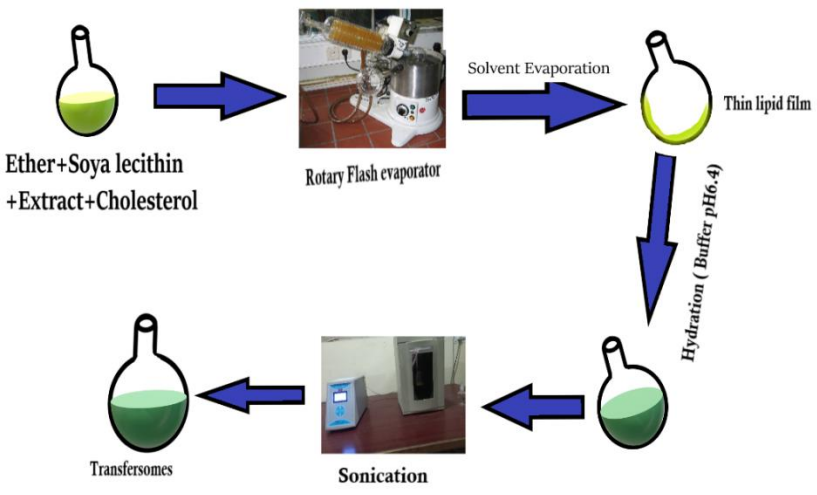

Figure 2. Schematic Representation of method

2.6.2. Formulation of gel

Transfersome was dissolved in DCM (Dichloromthane). Then CMC (Carboxi methyl cellulose) and Sodium Alginate was added into it. Buffer 6.4 pH and carbapol mix into it. It forms Transfersomal gel.

Table 2. Formulation of gel

S. No.	Chemical Name	Use (w/w)				
		F- 01	F- 02	F- 03	F- 04	F- 05
01	Transfersome (5mg/ml)	2 ml	2 ml	2 ml	2 ml	2 ml
02	DCM	10 ml	10 ml	10 ml	10 ml	10 ml
03	CMC	0.3 g	0.25 g	0.20 g	0.15 g	0.1 g
04	Sodium Alginate	100 mg	150 mg	200 mg	250 mg	300 mg
05	Charbapole	0.1 g	0.1 g	0.1 g	0.1 g	0.1 g
06	Phosphate Buffer 6.4 pH	10 ml	10 ml	10 ml	10 ml	10 ml

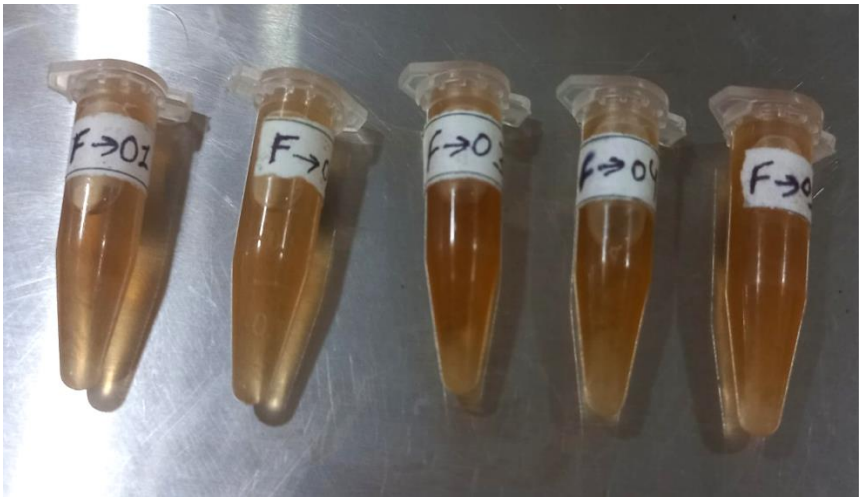

Figure 3. Formulations

3. Characterization of Transfersomes Gel

Determination of viscosity: Viscosities of the gels were determined by using Brookfield Viscometer. Spindle type, RV-7 at 20 rpm. 100 gm of the gel was taken in a beaker and the spindle was dipped in it and rotated for about 5 minutes and then reading was taken.

Extrudability: Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slides. Measuring the force needed to extrude the material from the tube is a helpful empirical test. The formulations were put into collapsible metal tubes with a 5 mm nasal tip whole. The quantity of gel that extruded from the tip of the tube when pressure was applied was used

to measure the extrudability of the tube. The formulation's extrudability was examined, and the outcomes were recorded.

Spredability: Gel was placed on the upper slides so that the gel was between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only upper slides to slip off freely by the force of weight tied on it. Weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated for three times and the mean time was taken for calculation. Spreadability was calculated by using the following formula:

$$S=M*L/T \quad (1)$$

In Vitro Drug Release

Diffusion Study for Transfersomes: Using the open-ended cylinder technique, the in vitro release of extract of *Tamarindus indica* from the Transfersomes formulations was investigated. The glass tube used in this diffusion cell device has an inner diameter of 2.5 cm and is open on both ends. One end is used as a donor compartment and is linked with an artificial membrane.

The purpose of this investigation is to ascertain the penetration rate. The formulations were optimized using data from in vitro tests and the time required to achieve permeation flow in a steady state. Using the in vitro diffusion technique, studies of drug release from transfersome gel formulations were conducted at 37 °C and 100 rpm for duration of 24 hours. Poured into the glass cell, a weighed quantity of the manufactured transfersome gel formulation was allowed to diffuse against phosphate buffer pH 6.4, which served as the diffusion medium. Using phosphate buffer pH 6.4 as a blank, aliquots were obtained at regular intervals and subjected to spectrophotometric analysis at 337 nm.

Examining the anti-inflammatory properties using the rat paw edema technique caused by carrageenan: The anti-inflammatory activity of formulated Transfersomes was carried out by using Albino rats as an animal model. Healthy albino male rats were selected.

- Group 1 served as a control.
- Group 2 served as a blank and received carrageenan and gel without a drug.
- Group 3 served as a standard and received carrageenan and diclofenac gel.
- Group 4 served as a test, which received carrageenan and formulated Transfersomes.

In the present study, approximately half an hour was spent preparing one suspension of CRR Gee. Before the experiment, it was injected into the rat's right hind paw's plantar area. 0.2 gm of F-02 were applied to the test groups' right hind paws' plantar surfaces and lightly rubbed with the index finger. The gel base was the only treatment given to the rats in the control group; the same procedure was followed when applying the standard gel, Diclofenac. Following an hour of gel base application, a topical preparation of F-02, consisting of 1 suspension of carrageenan

in saline, was applied to the rat's right hind paw's plantar area. Using a plethysmometer, paw volume was measured immediately following carrageenan injection at one, two, three, and four hours. The paw volume was measured at various intervals [16]. Using the following formula, the percentage inhibition in paw volume was determined:

$$\% \text{ Inhibition} = \frac{\text{Paw volume (control)} - \text{Paw volume (test)}}{\text{Paw volume (control)}} \times 100 \qquad (2)$$

Stability Study: Three groups of the prepared Transfersomal gels were formed. These three Transfersomal gel formulation groups were placed within collapsible aluminum tubes and kept at:

A. Temperature of the room (25 ℃);

B. 40 ℃;

C. 4 ℃.

For three months, the Transfersomal gel formulation was kept in storage. For duration of three months, samples were taken out each month and their drug content evaluated. They were assessed for physical parameters and product integrity at the conclusion of the third month.

Physical evaluation: The physical factors that were taken into account for the assessment were the product's nature, extrudability, pH, viscosity, leak, and phase separation.

4. Results and Discussion

4.1. Pre-formulation study

4.1.1. Phytochemical study of *Tamarindus indica* extract

Table 3. Phytochemical of *Tamarindus indica* extract

S.No.	Test Name	Result
01	Alkaloids	+
02	Flavonoids	-
03	Saponins	+
04	Steroids	+
05	Glycosides	+
06	Monosaccharaides	+
07	Carbohydrates	+
08	Proteins	+
09	Mucilage & Gums	+
10	Terpenoids	-

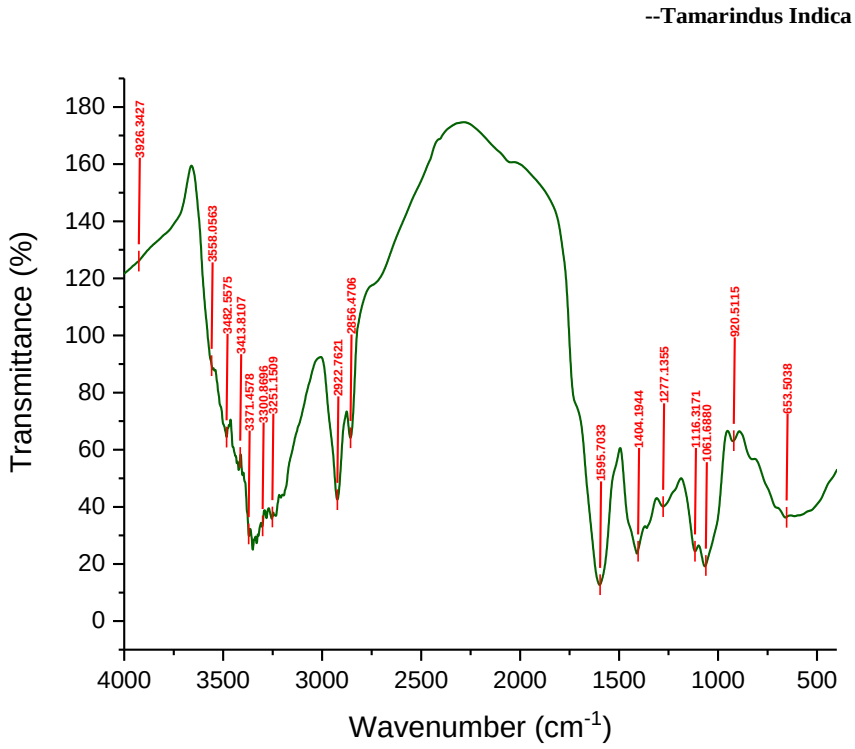

Figure 4. FT-IR of *Tamarindus indica* extract

Table 4. FT-IR interpretation of *Tamarindus indica* extract

S.No.	Peak Position	Group
01	3482.5575	O-H stretching, N-H stretching
02	3413.8107	O-H stretching
03	3371.4578	O-H stretching, N-H stretching
04	3300.8696	O-H stretching, N-H stretching, C-H stretching
05	3251.1509	O-H stretching
06	2922.7621	O-H stretching, N-H stretching, C-H stretching
07	2856.4706	O-H stretching, N-H stretching, C-H stretching
08	1595.7033	N-H bending, C=C stretching
09	1404.1944	C-H bending, O-H bending, S=O stretching
10	1277.1355	C-F stretching, C-N stretching, C-O stretching
11	1116.3171	C-F stretching, C-N stretching, C-O stretching
12	1061.6880	C-F stretching, C-N stretching, C-O stretching, S=O stretching
13	653.5038	C-Cl stretching, C-Br stretching

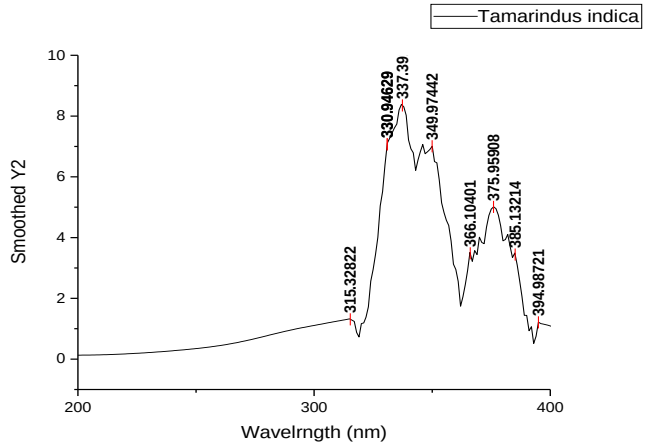

Figure 5. UV of *Tamarindus indica* extract

4.1.2. Drug-Excipients interaction study

- The drug plant extract and the excipients namely Soya lecithin, carbopol, Cholesterol were analyzed by FT-IR spectrophotometer.

- The FT-IR spectrums were interpreted and it is shown there is no interaction between the drugs with the excipients was conformed.

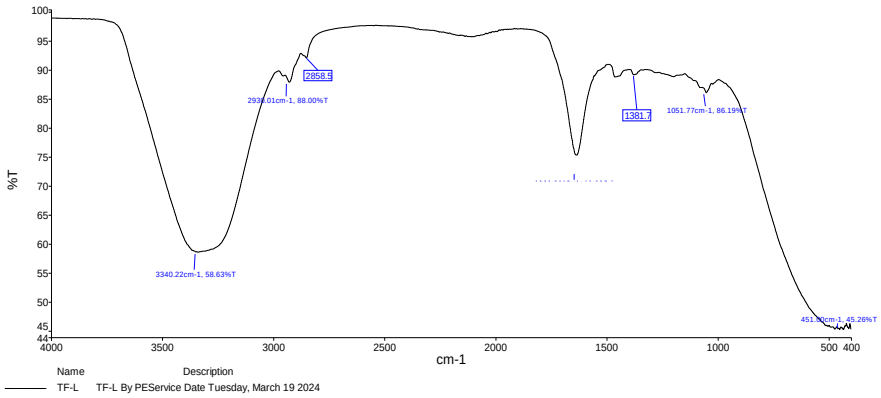

Figure 6. FT-IR of Tf L

Table 5. FT-IR interpretation of Tf L

S.No.	Peak Position	Group
01	3340.22	O-H stretching, N-H stretching
02	2930.01	O-H stretching, N-H stretching, C-H stretching
03	2858.5	O-H stretching, N-H stretching
04	1635.020	C=O stretching, C=C stretching, N-H bending
05	1381.7	C-H bending, O-H bending, S=O stretching, C-F stretching
06	1051.77	C-F stretching, C-N stretching, C-O stretching, S=O stretching

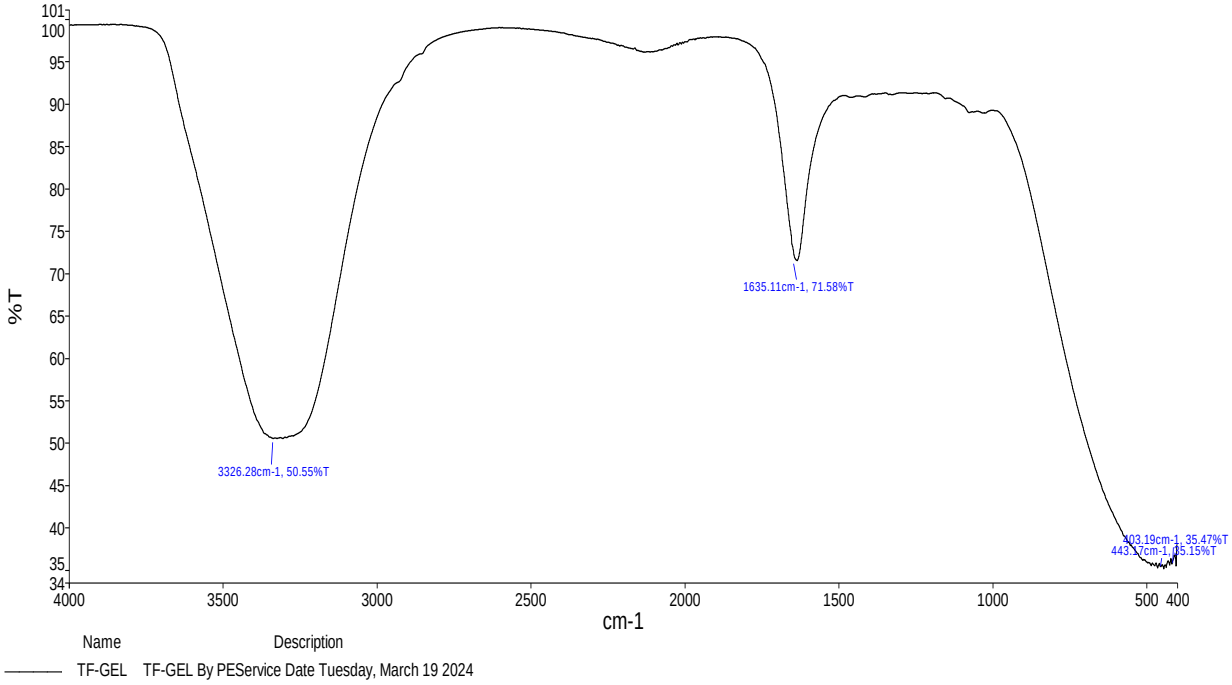

Figure 7. FT-IR of Tf Gel

Table 6. FT-IR interpretation of Tf Gel

S.No.	Peak Position	Group
01	3326.28	O-H stretching, N-H stretching, C-H stretching
02	1635.11	C=O stretching, C=C stretching, N-H bending

4.1.3. Solubility Studies

Table 7. Solubility Test

S.No	Chemical Name	Result
01	Methanol	+++
02	Ethanol	++++
03	Ether	++++
04	Water	++

4.2. Characterization of Transfersomes

- **Particle Size Analysis of Transfersomes**

The Transfersomes were subjected to microscopic examination (S.E.M) for characterizing size and shape of the Transfersomes. Microscopic examination revealed, spherical small uni-lamellar vesicles of 360 to 550 size range. The average mean particle size of formulation-2 was 455 nm, respectively. Photographs were given in Figures 8-9.

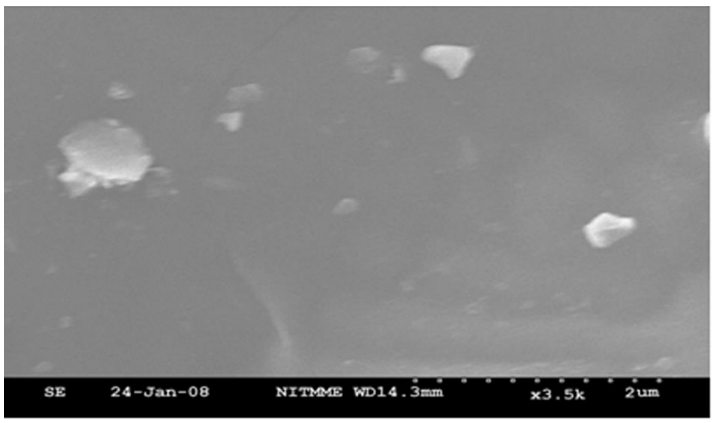

Figure 8. Particle Size and shape of Transfersomes Gel

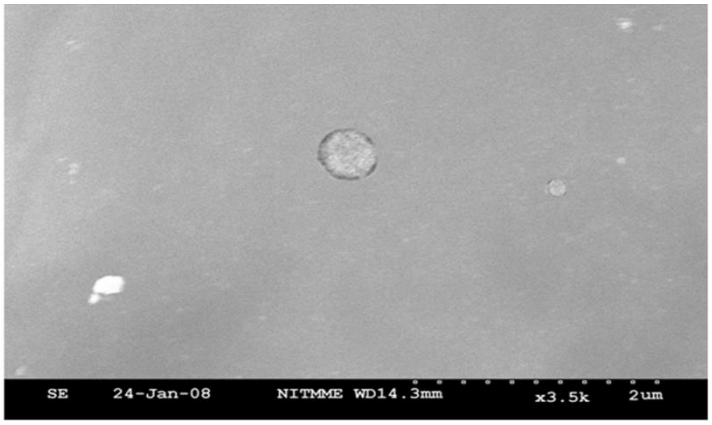

Figure 9. Particle Size and shape of Transfersomes

4.3. Transfersomes Gel Evaluations

4.3.1. Determination of viscosity

The viscosity of the gels was determined by using Brookfield viscometer. The viscosity of the formulations were ranged from 46,000 to 50,000 cps and the results were shown in Table no. 8.

Table 8. Viscosity Test

S. No.	Formulation	Viscosity in cps
01	Formulation-1	48,000
02	Formulation-2	48,500
03	Formulation-3	46,994
04	Formulation-4	47,980
05	Formulation-5	48,459

4.3.2. Extrudability

The extrudability of the gel formulations were checked as per the procedure, extrudability of gels was excellent. It is shown in Table no. 9.

Table 9. Extrudability Test

S. No.	Formulation	Extrudability
01	Formulation-1	+++
02	Formulation-2	+++
03	Formulation-3	+++
04	Formulation-4	++
05	Formulation-5	++

4.3.3. Spredability

Table 10. Spredability Test

S. No	Formulation	Result
01	Formulation-01	20±31
02	Formulation-02	25±31
03	Formulation-03	22±31
04	Formulation-04	23±31
05	Formulation-05	21±31

4.3.4. In Vitro Drug Release

Diffusion Study for Transfersomes

Table 11. Drug Release Test

S. No.	Time in (hrs.)	Absorbance at 337 nm
1	0	0
2	0.25	0.627
3	0.5	0.648
4	0.75	0.656
5	1	0.659

6	1.5	0.662
7	2	0.668
8	2.5	0.669
9	3	0.671
10	4	0.674
11	5	0.678
12	6	0.686
13	7	0.688
14	8	0.689
15	9	0.694
16	10	0.696
17	11	0.698
18	12	0.699
19	14	0.703
20	16	0.709
21	18	0.715
22	20	0.717
23	22	0.718
24	24	0.718

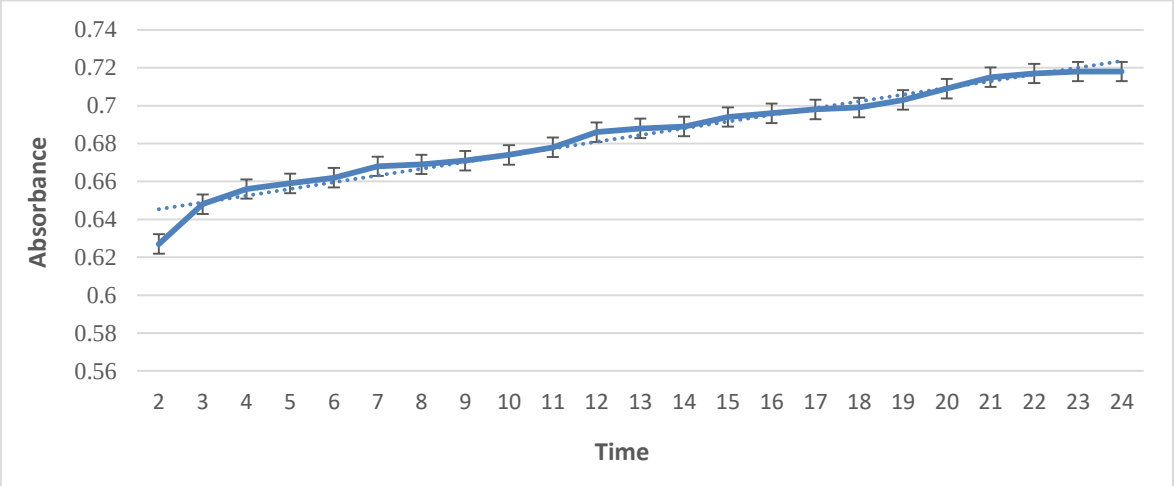

Figure 10. Drug Release Test

4.3.5. Anti-inflammatory properties using the rat paw edema technique caused by carrageenan

Table 12. Animal Testing

School of Pharmacy, ITM University, Gwalior (Animal Testing).									
Sheet Name	**Pradyumn Tiwari**								
Sample Name	**Transfersomal Gel**				**Species of Animal**	**Rat**			
B.No.	**F-02**				**Gender of Animal**	**Male**			
Dose	**ST- 1 ml, GB- 1 ml, ST- 1 ml, TTFI- 1 ml**				**Drug Given**	**ST- 0.039 mg/ml, TTFI- 2 mg/ml**			
Test Activity	**Anti-inflammatory Effect**								
Study Title	**Formulation and Evaluation of *Tamarindus indica* Extract Loaded Transfersomal Gel for Anti-inflammatory Effect**								
Grouping of Animals									
Group	**Gender**	**Dose**	**Animal ID**	**Initial Body Weight (gm)**	**Group**	**Gender**	**Dose**	**Animal ID**	**Initial Body Weight (gm)**
Group -1	Male	NA	Pg1 - 1M	100	**Group - 3 [ST + CR] (0.039 gm /ml)**	Male	1 ml	Pg3 - 1M	125
	Male	NA	Pg1 - 2M	150		Male	1 ml	Pg3 - 2M	150
	Male	NA	Pg1 - 3M	175		Male	1 ml	Pg3 - 3M	150
	Male	NA	Pg1 - 4M	164		Male	1 ml	Pg3 - 4M	135
	Male	NA	Pg1 - 5M	149		Male	1 ml	Pg3 - 5M	165
	Male	NA	Pg1 - 6M	135		Male	1 ml	Pg3 - 6M	125
Group - 2 (GB + CR)	Male	1 ml	Pg2 - 1M	125	**Group - 4 [TTFI + CR] (02 mg /ml)**	Male	1 ml	Pg4 - 1M	175
	Male	1 ml	Pg2 - 2M	175		Male	1 ml	Pg4 - 2M	150
	Male	1 ml	Pg2 - .3M	100		Male	1 ml	Pg4 - 3M	100
	Male	1 ml	Pg2 - 4M	162		Male	1 ml	Pg4 - 4M	125
	Male	1 ml	Pg2 - 5M	149		Male	1 ml	Pg4 - 5M	135
	Male	1 ml	Pg2 - 6M	146		Male	1 ml	Pg4 - 6M	125
Symbol									
NC (Group-1)	**Normal Control**								
GB	**Gel without drug**								

NA	**Blank**
CR	**Carrageenan**
ST	**Standard (Diclofenac gel)**
TTFI	**Test of *Tamarindus indica* Extract Loaded Transfersomal Gel**
Dose	
Standard (Diclofenac Gel)	**0.039 gm/ml**
Transfersomal Gel	**02 mg/ml**

Table 13. Animal Testing Result

S. No.	Time (h)	% Inhibition of edema		
		Control (without drug)	Standard (Diclofenac gel)	Test (F-02)
01	01	21.21	33.12	25.32
02	02	24.33	60.51	59.09
03	03	33	77.57	76.03
04	04	36.21	95.23	86.94

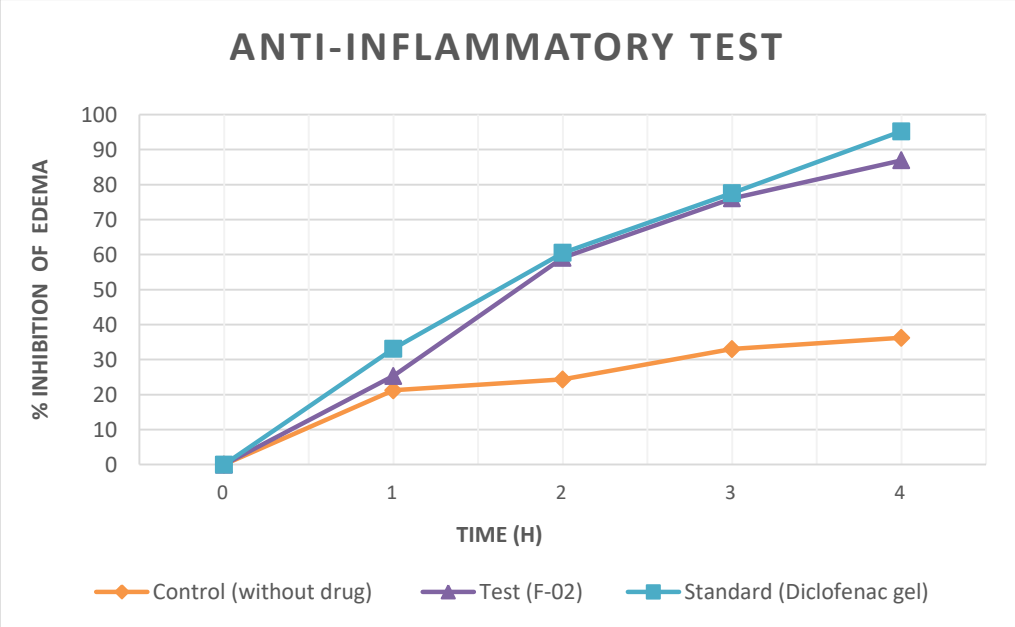

Figure 11. Animal Testing Result

4.3.6. Stability Study

The stability studies of Transfersomal formulation were carried out at refrigeration temperature (4 ℃), Room temperature and 40 °C. Physical evaluation of prepared Transfersomes gel shown in the Table no. 14.

Table 14. Stability Study

Parameter	Room Temperature (25 °C)	40 ^{0}C	4 ^{0}C
Visual Appearance			
• **Initial**	Brown colour gel	Brown colour gel	Brown colour gel
• **1 month**	Brown colour gel	Brown colour gel	Brown colour gel
• **2 month**	Brown colour gel	Brown colour gel	Brown colour gel
• **3 month**	Brown colour gel	Brown colour gel	Brown colour gel
pH			
• **Initial**	6.7	6.7	6.7
• **1 month**	6.7	6.7	6.7
• **2 month**	6.8	6.8	6.8
• **3 month**	6.8	6.8	6.8
Viscosity			
• **Initial**	48,500	48,500	48,500
• **1 month**	48,500	48,500	48,500
• **2 month**	48,497	48,497	48,500
• **3 month**	48,495	48,495	48,497
Extrudability			
• **Initial**	Satisfactory	Satisfactory	Satisfactory
• **1 month**	Satisfactory	Satisfactory	Satisfactory
• **2 month**	Satisfactory	Satisfactory	Satisfactory
• **3 month**	Satisfactory	Satisfactory	Satisfactory
Phase Separation			
• **Initial**	Not found	Not found	Not found
• **1 month**	Not found	Not found	Not found
• **2 month**	Not found	Not found	Not found
• **3 month**	Not found	Not found	Not found

Texture			
• **Initial**	Smooth	Smooth	Smooth
• **1 month**	Smooth	Smooth	Smooth
• **2 month**	Smooth	Smooth	Smooth
• **3 month**	Smooth	Smooth	Smooth

5. Conclusion

The phytochemical and pharmacological evaluation of trnasfersome formulations of *Tamarindus indica* powder extract demonstrates their potential as promising therapeutic agents. These formulations offer enhanced bioavailability and pharmacological activity compared to conventional extracts, making them valuable candidates for further development and clinical investigation. The synergistic interactions between *Tamarindus indica* constituents and phospholipids in the trnasfersome formulation contribute to improved absorption and targeted delivery, enhancing their efficacy in various therapeutic applications. Future research should focus on elucidating the mechanisms of action, optimizing formulation parameters, and conducting clinical trials to validate the efficacy and safety of trnasfersome formulations of *Tamarindus indica* in human subjects. Overall, this study underscores the importance of innovative formulation approaches in harnessing the therapeutic potential of herbal medicines like *Tamarindus indica* for improved healthcare outcomes.

6. Future Recommendations

Clinical Trials and Human Studies: Conduct extensive clinical trials to evaluate the safety, efficacy, and tolerability of *Tamarindus indica* extract-loaded transferosomal gel in human subjects. These studies will help confirm its potential as an anti-inflammatory treatment and determine optimal dosage regimens.

Mechanistic Studies: Investigate the underlying molecular mechanisms of the anti-inflammatory effects of the *Tamarindus indica* extract. Understanding the specific pathways and targets involved can help refine the formulation and enhance its therapeutic efficacy.

Comparative Studies with Existing Treatments: Compare the efficacy and safety of the *Tamarindus indica* extract-loaded transferosomal gel with existing anti-inflammatory treatments. These comparative studies can establish its relative advantages, such as reduced side effects, improved patient compliance, or enhanced potency.

Formulation Optimization: Explore further optimization of the transferosomal gel formulation to enhance drug delivery, stability, and patient acceptability. This might include tweaking the composition of the Transfersomes, adjusting the gel matrix, or incorporating additional stabilizers or enhancers.

Expansion to Other Therapeutic Areas: Investigate the potential of the *Tamarindus indica* extract-loaded transferosomal gel in treating other inflammatory conditions beyond its initial application. This could include exploring its use in dermatological conditions, musculoskeletal disorders, or systemic inflammatory diseases, broadening the scope and impact of the formulation.

Declarations

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Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this study.

Ethical Approval

Based on institutional guidelines.

Authors' contributions

All the authors took part in literature review, analysis and manuscript writing equally.

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